reciprocal plots to provide k_c and K_m. Calculations of second-order rate constants k_2', equal to k_c/K_m, were by the method of least squares. Initial velocities of saturation kinetics runs were measured directly from the recorded optical density *vs.* time curves. All polynomial and least-squares computations employed an IBM 360/40 computer. Table I displays the k_c, K_m, and k_c/K_m values ultimately obtained. The rates of hydroxide ion catalyzed hydrolysis of most *p*-nitrophenyl esters were determined at 400 nm in 20% methanolic carbonate–bicarbonate buffers of pH 9–11.[47] *p*-Nitrophenyl esters of amidotetrahydro, amidoindan, and (*S*)-AP were more conveniently hydrolyzed at pH 8 in 20% methanolic phosphate buffer (0.067 *M*). Reaction mixture and buffer pH values were measured on a Brinkman E 300B meter. First-order rate constants obtained from the half-lives of the hydrolyses were divided by apparent hydroxide ion concentrations to give the second-order rate constants which were used to obtain the relative reactivities of Table II *p*-nitrophenyl esters.

Hydroxide ion catalyzed hydrolysis rates of (*S*)-APME, tetrahydro methyl ester, indan methyl ester, and amidoindan methyl ester were measured in a Brinkman Model 3D combititrator equipped with a 1-ml buret, thermostated reaction vessel, and type H electrode. Hydrolyses were carried out at 25° by adding to 20 ml of 30% DMSO–water, 0.1 *M* in sodium chloride, 1 ml of DMSO containing the appropriate esters. Duplicate runs were obtained at ester concentrations of 0.8 and 1.6 m*M*. The system was purged with nitrogen, the pH brought to 11.4 by addition of concentrated sodium hydroxide, and the uptake of 0.05 *M* sodium hydroxide was recorded as a function of time. The observed first-order rate constants were obtained by the half-lives method from plots of $V_\infty - V_t$ *vs.* time. The following first-order rate constants (in $sec^{-1} \times 10^4$) were obtained from the linear plots through 3 half-lives: (*S*)-APME, 7.01; tetrahydro methyl ester, 1,13; indan methyl ester, 1.59; amidoindan methyl ester, 0.66. Acetoxyindan methyl ester took up 2 equiv of base in a curve with no clean break; therefore, its reactivity and that of acetoxytetrahydro methyl ester were estimated. Amidotetrahydro methyl ester was hydrolyzed too slowly to be measured on the pH-stat and a manual titrimetric procedure was used.

To 200 ml of a 30% DMSO–water 0.1 *M* in sodium chloride and 0.0105 *M* in sodium hydroxide equilibrated at 25° under nitrogen was added 10 ml of DMSO containing 2.1 mmol of the substrate, making the final concentration of each reactant 0.01 *M*. At appropriate intervals 10-ml aliquots were withdrawn and added to flasks containing 10 ml of 0.015 *N* potassium acid phthalate to quench the reaction. The excess phthalate was titrated against 0.01 *N* sodium hydroxide to the phenolphthalein end point. A second-order treatment[48] gave a straight line and a rate constant of $7.22 \times 10^{-3}\ M^{-1}\ sec^{-1}$ was calculated from the slope. Using the same procedure the second-order rate constant for tetrahydro methyl ester was $1.25 \times 10^{-1}\ M^{-1}\ sec^{-1}$. A relative reactivity scale in which tetrahydro methyl ester was arbitrarily assigned a standard value of 100 was set up using the values obtained by the two methods (Table II).

Acknowledgment. We wish to thank Dr. Marc S. Silver for helpful comments. This research was supported by the National Institutes of Health (GM 18652), a National Science Foundation Institutional Grant, and the Graduate School, Southern Illinois University at Edwardsville.

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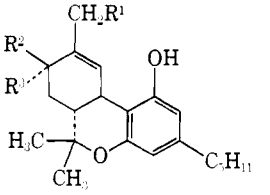
Communications to the Editor

Synthesis of 11-Hydroxy-Δ^9-tetrahydrocannabinol and Other Physiologically Active Metabolites of Δ^8- and Δ^9-Tetrahydrocannabinol

Sir:

Studies[1a-e] using laboratory animals have established that Δ^9-tetrahydrocannabinol (Δ^9-THC, **1a**), the principal psychotomimetic constituent of marihuana and hashish,[2] is readily metabolized to a number of hydroxylated metabolites. One of these, 11-hydroxy-Δ^9-THC (**1b**), has been identified in man[3a-d] and shown to have high physiological activity.[4a-c] Recent studies[3a,5] in our laboratories have revealed that Δ^9-THC is, in addition, partially metabolized in man to 8α- and 8β-hydroxy-Δ^9-THC (**1c**, **1d**), and these metabolites



1a, $R^1 = R^2 = R^3 = H$
b, $R^1 = OH$; $R^2 = R^3 = H$
c, $R^1 = R^2 = H$; $R^3 = OH$
d, $R^1 = R^3 = H$; $R^2 = OH$

also exhibit some degree of activity.[3a,6] These findings, and the urgent need for these metabolites in the further elaboration of the pharmacology of marihuana, prompt us to report a simple, essentially one-step synthesis which provided all three metabolites, and represents the first, albeit low yield, practical source of 11-hydroxy-Δ^9-THC.[7]

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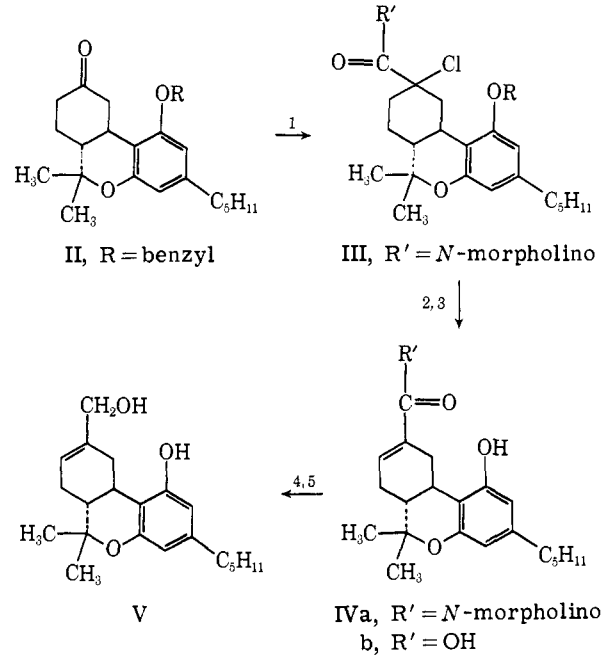
(7) Previously 11-hydroxy-Δ^9-THC has only been available through microsomal hydroxylation,[1a] a tedious and expensive method when applied preparatively. Chemists have made many unsuccessful attempts to synthesize this compound, the only published report[8] involving selenium dioxide oxidation of Δ^9-THC. The low yield (1%) and

The synthesis involves allylic halogenation of Δ^9-THC acetate with sulfuryl chloride, followed by acetoxylation with silver acetate. Thus, redistilled sulfuryl chloride (1 mol equiv) was added to a stirred solution of Δ^9-THC acetate (8.7 g) in carbon tetrachloride (80 ml) at room temperature over a period of 5 min. After approximately 60 min (glc showed quantitative reaction), the solvent was evaporated *in vacuo* and replaced by 1.4 *M* silver acetate in acetic acid (1.5 mol equiv). After 24 hr, the mixture of crude acetoxylated products was isolated and saponified with aqueous ethanolic potassium hydroxide at room temperature (16 hr). Gradient elution from silica gel with an acetone–benzene mixture first afforded 8β-hydroxy-Δ^9-THC (1.2 g, 14%), followed by 8α-hydroxy-Δ^9-THC (80 mg, 1%), and finally 11-hydroxy-Δ^9-THC (415 mg, 5%). The 8β- and 11-hydroxylated products were spectroscopically identical with authentic samples,[1a-b,5] 8α-Hydroxy-Δ^9-THC was identified on the basis of its high-resolution mass spectrum [*m/e* 330.219 (calcd for $C_{21}H_{30}O_3$, 330.219), 315 (M − CH_3), 312 (M − H_2O), 311 (M − H_2O–H), 297 (M − H_2O–CH_3), 231 (M − H_2O–C_6H_9)] and comparison of the nmr spectrum with published data.[9]

A number of other allylic halogenating and oxygenating reagents[10] have been examined, with the object of exploiting any variation in selectivity of attack at the primary and secondary allylic sites of Δ^9-THC. *N*-Bromo- and *N*-chlorosuccinimide, triethylcarbinyl hypochlorite, and molecular chlorine all produce predominantly 8β-hydroxy-Δ^9-THC after acetoxylation and saponification, with only minor amounts of 11-hydroxy-Δ^9-THC. Palladium acetate[11] and *tert*-butyl peracetate afford only cannabinol. The use of 2 equiv of sulfuryl chloride leads to reduced yields of the monohydroxy metabolites and no 8,11-dihydroxy-Δ^9-THC, apparently because of competing aromatic chlorination after the introduction of the first chlorine atom. The very efficient[1] conversion of Δ^9-THC to 11-hydroxy-Δ^9-THC *via in vitro* microsomal enzymatic hydroxylation emphasizes the uniqueness of the biochemical mechanism.

An alternate, more lengthy, synthesis of 11-hydroxy-Δ^9-THC failed because of unexpected loss of stereochemical control, but instead provided a new[12] route to 11-hydroxy-Δ^8-THC (V, Scheme I), a physiologically active metabolite of Δ^8-THC. Thus, preparation of the α-chloramide III from the reaction of the morpholino enamine of 11-nor-9-ketohexahydrocannabinol *O*-benzyl ether[13] (II) with trichloroacetic acid,[14] followed by cleavage of the benzyl protecting group and conversion to the phenoxide anion to effect elimination of HCl, gave only the Δ^8-amide (IVa). Despite precedent,[15] no Δ^9-amide, which would be derived from intramolecularly assisted elimination, was formed. Saponification of IVa afforded the carboxylic acid IVb which, when reduced with lithium aluminum hydride, gave 11-hydroxy-Δ^8-THC in *ca.* 33% overall yield. This synthetic approach is likely to be of value because of the belief[16] that carboxylic acids related to IVb are involved in the general metabolic degradation of tetrahydrocannabinols.

Scheme I. Conversion of 11-Nor-9-ketohexahydrocannabinol to 11-Hydroxy-Δ^8-THC[a]



[a] Reagents: 1, morpholine–trichloroacetic acid; 2, HCl–$ZnCl_2$·$2H_2O$–$CHCl_3$; 3, Et_3COK–toluene, 0°; 4, aqueous EtOH–KOH; 5, $LiAlH_4$.

Acknowledgments. This work was carried out under Contract No. HSM-42-71-108, of the National Institute of Mental Health, National Institutes of Health. Mass spectral data were obtained at the Research Triangle Institute Mass Spectrometry Center under National Institutes of Health Grant No. PO7 RR-00330-05.

the difficulty of removing all toxic selenium makes this impractical for pharmacological purposes. Other less direct, individual syntheses of 8α- and 8β-hydroxy-Δ^9-THC have recently been reported.[6,9]

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Identification of Δ^9-Tetrahydrocannabinol and and Metabolites in Man

Sir:

In recent years there has been unprecedented interest in the chemistry, metabolism, and pharmacology of